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Chapter 41

Aquacultured coral and restoration

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ABSTRACT
The Coral Reef Task Force estimates that 70 % of the world's coral reefs are threatened and 10 % have been destroyed. Portions of Caribbean coral reefs have lost up to 80 % of coral species and continue to be under increasingly destructive pressures from various sources, including dredging, ship groundings, pollution, illegal collecting and harsh weather conditions. Florida's coral reefs, the only shallow water reefs in the continental United States, have suffered considerable loss. Restoration of damaged coral sites is limited by the availability of coral colonies. Aquaculture is emerging as a viable method of large-scale production of coral colonies (especially Indo-Pacific species) using fragmentation for the aquarium trade. Recent efforts have shown that many species of Atlantic Scleractinia can be fragmented and grown successfully in tanks and on underwater lease sites. Can these aquacultured fragments be utilized in reef restoration? Two primary questions emerge concerning the feasibility and direction of this effort: i) will aquacultured corals become a vector for disease introduction when returned to a restoration site, and ii) is the survival and growth success of reintroduced fragments affected by culture techniques? The research outlined in this paper will provide information on techniques and protocols to help answer these questions and improve coral restoration efforts.

INTRODUCTION

Florida is the only state in the continental United States that has extensive shallow coral reef formations near its coasts, from the Florida Keys starting south of Miami reaching west to the Dry Tortugas. Caribbean coral reefs are under increasingly destructive pressures from various sources, including dredging, ship groundings, pollution, and illegal collecting of organisms. The state's Comprehensive Conservation Wildlife Strategy of 2005 cited coral reefs as a priority habitat, labeled "bad and in decline." Over 200 species of birds, mammals, fish and invertebrates, including numerous coral species, were designated "species of greatest conservation need."

At The Florida Aquarium, our involvement with these reefs is multi-factorial and growing. Our conservation projects are chosen on the basis of challenges facing Florida aquatic ecosystems and utilize the expertise of The Florida Aquarium's staff. The projects focus on husbandry practices and improving the health of captive populations as well as contributing to field studies that assess and advance the health of Florida ecosystems. Additional selection criteria include opportunities for public education regarding Florida's critical conservation issues and the Aquarium's work through exhibits and programming. Finally, key to all our efforts, we strive to facilitate cooperative efforts with local research and educational facilities as well as national and foreign organizations.

The roles of the Florida Keys National Marine Sanctuary (FKNMS) and the Florida Fish and Wildlife Conservation Commission (FWC) in this project further highlight the importance of partnerships. In 1990 the US Congress designated the Florida Keys National Marine Sanctuary which is administered by the National Oceanic and Atmospheric Administration (NOAA) in partnership with the Florida Department of Environmental Protection (FDEP). The Sanctuary extends approximately 220 miles southwest from the southern tip of the Florida peninsula and encompasses 2,800 square nautical miles of the waters surrounding the Florida Keys It contains nationally significant marine environments, including seagrass meadows, mangrove islands, and extensive living coral reefs. The overall goal of the Sanctuary is to balance the long-term health of the ecosystem with the economy it supports. The Florida Wildlife Legacy Initiative Grants, administered through the Florida Fish and Wildlife Conservation Commission, are the result of an action plan for conserving all of the state's wildlife and vital natural areas for future generations. It is part of a nation-wide effort by all 50 states and 6 U.S. territories to develop action plans and qualify for federal funding. Completed in September 2005, the Strategy was approved by the U.S. Fish and Wildlife Service in December 2005, and it outlines which Florida native wildlife and habitats are in need, why they are in such a condition and what needs to be done.. The motto for the Florida program is to "Keep Common Species Common," and the goal is to prevent wildlife from becoming endangered before they become more rare and costly to protect. Also, rather than rules and regulations, the Initiative encourages partnerships to take action. The coral project at The Florida Aquarium highlights many of the goals from these two organizations.

The specific objectives of the coral project at The Florida Aquarium include: i) construct a land-based propagation facility at FLAQ and use it to educate visitors, ii) produce fragments for exchange and research, iii) serve as a holding facility for damaged corals and orphaned corals (corals obtained from dock repair projects and permitted through the FKNMS Reef Medic and Coral Nursery programs), iv) conduct cooperative research on captive coral health and propagation issues, and v) develop restoration projects and health certification protocols.

In 2000-2001, through funding from the Association of Zoos and Aquariums' (AZA) Conservation Endowment Fund (CEF) and NOAA's 5 Star Restoration Grants Program, The Florida Aquarium built a working propagation facility, the "Coral Farm" on the exhibit pathway (Figure 1). Educational brochures which describe the project are available to the public and additional programming tells the message through outreach programs, camps and teacher workshops (part of the Science Education at Sea (SEAS) program). There is even a website camera focused on a coral fragment in the farm

Figure 1: The Florida Aquarium's coral propagation facility, "Coral Farm" on the exhibit pathway.

to watch corals grow! By revisiting the site over several months, the public learns how slowly these animals grow and how difficult it can be to restore damaged sites.

Our research studies at The Florida Aquarium initially were focused on exploring propagation methods in culture situations and evaluating requirements such as lighting, flow, attachment, size, compatibility, among other factors. Over time, additional projects emerged including delineating diseases in "captivity", developing diagnostic tools and treatments for disease, and propagating sufficient "lab rats" for cooperative research.

However, key questions remained unanswered. Could these fragments ever be reintroduced back to the wild? and, if they could, was there a potential for larger scale production in aquaculture facilities to provide adequate numbers of fragments for restoration projects? With support from the FKNMS and a Florida Wildlife Legacy Initiative grant (#SWG04-038) through the FWC, The Florida Aquarium and partners began addressing two primary questions concerning the use of aquacultured fragments for restoration: i) would culture techniques affect survival and growth of reintroduced fragments and ii) could these fragments become a vector for disease when returned to a restoration site? As the study advanced, many additional questions arose that could impact the successful use of aquacultured corals to rehabilitate reefs. What species should be collected and recovered? What were appropriate culture parameters? Was there a way to mitigate potential for introduction of disease? What were geographical and genetic concerns? And what about over-riding environmental concerns such as climate change? To help focus future studies, the initial partnership expanded to include additional coral restoration stakeholders from around the state. To help guide future studies, the working group, known as the Florida Cultured Coral Conservation Consortium (The Florida C's,) adapted a ten point list from Blankenship and Leber (1995) on a reasonable approach to marine stock enhancement. In sum, the ten points include: 1) prioritize species, 2) identify harvest and genetic objectives, 3) define quantitative measures of success, 4) avoid inbreeding, 5) include disease and health management, 6) consider ecological, biological life-history patterns, 7) assess stocking impact, 8) identify optimum release protocols, work with pilot releases to help define them, 9) identify economic and policy guidelines, and 10) use adaptive management concepts (continually revisit and revise).

PROJECT OVERVIEW

Corals for this study were made available by the FKNMS. Over a period of several years, during the construction activities at the Truman Annex Mole Pier in Key West, Florida, over 3,500 corals and fragments from this one site were removed. Disposition choices included immediate transplantation to restoration sites, donation to aquariums for exhibits or donation for research.

In brief, the culturing portion of the study was to compare growth and survival between two land-based aquaculture facilities with an open-water site. In an attempt to design economically feasible culture techniques (for which cost-benefit analyses would later be run), culture methods were relatively basic in design. The first land-based site was at the Tropical Aquaculture Laboratory, University of Florida, in Ruskin, Florida (Figure 2). Corals were maintained in a commercial style of greenhouse (9.1 m x 22 m) with inflated double layer poly 30 % shade cloth) with fan shutters and a propane heater. Fragments were placed in two 1,325 liter tanks, each 3 m x 0.75 m x 0.75 m , and elevated on racks constructed of PVC pipe and plastic lighting grids ("egg crate"). Water was supplied to each tank utilizing a "Carlson" surge generator constructed from plastic drums and PVC pipe. The system included a sump that measured 2 m x 1 m x 0.75 m, filled with approximately 20 cm of crushed coral, which served as a calcium source and assisted with biological filtration. The system was powered by a 1.0 HP centrifugal pump. The system also included a 0.5 ton water chiller. During the winter months, the greenhouse was heated. Temperatures were maintained at 25-27ºC. It was a closed water system, using artificial seawater made by combining reverse osmosis water with Instant Ocean® sea salt mixture to achieve a salinity of 33 ppt. Water changes of at least 50 % were made once each month. The culture tanks were also covered with a PVC frame and 50 % shade cloth. The second land-based site was at the Mote Marine Laboratory on Summerland Key (Figure 3). The tank set-up was similar but water was obtained directly from the inlet (on open-flow system). The field/open-water site (control site) was the Miss Beholden grounding

Figure 2: The greenhouse culture system at the Tropical Aquaculture Laboratory, University of Florida.

Figure 3: Open-flow system at the Mote Marine Laboratory on Summerland Key.

Figure 4: The Miss Beholden grounding site on Western Sambo Reef, the current restoration site.

site, on Western Sambo Reef, approximately 4 nautical miles south of Key West (Figure 4).
Project corals were obtained from the Truman Annex site in May of 2006. Seven species were utilized; *Siderastrea radians*, *Solenastrea bournoni*, *Montastrea annularis*, *Montastrea cavernosa*, *Diploria clivosa, Dichocoenia stokesii*, and *Stephanocoenia mechelinii.* No branching species were available from this site. Coral colonies were selected for size and suitability for fragmentation. Genetically identical colonies were preferred but not available for all species due to size of parent colony stock. Where multiple parent colonies were used, each of the three facilities received representative samples. 30 fragments of each species were made to allow for 10 fragments per culture site. The corals were fragmented, using a tile saw with sea water as a coolant, into approximately 2.5 x 2.5 cm pieces (Figure 5). Using a two part epoxy (Z-Spar®), each fragment was affixed to a standardized cement base, 8.5 cm in diameter and 1.5 cm thick. (Figure 6) Ten fragments of each species were distributed to each of the three sites (Figure 7). The fragments were to be held in culture for a minimum of 6 months, during which time periodic (a minimum of every three months) health assessments and disease diagnostics were performed. The field site is to be evaluated every three months (Figure 8). Digital images were taken of each fragment with a scale bar

Figure 5: Cutting coral colonies into fragments using a tile saw with sea water as a coolant. Measuring individual pieces.

Figure 6: Attaching the coral fragment to a concrete base using a 2-part epoxy, Z-Spar®.

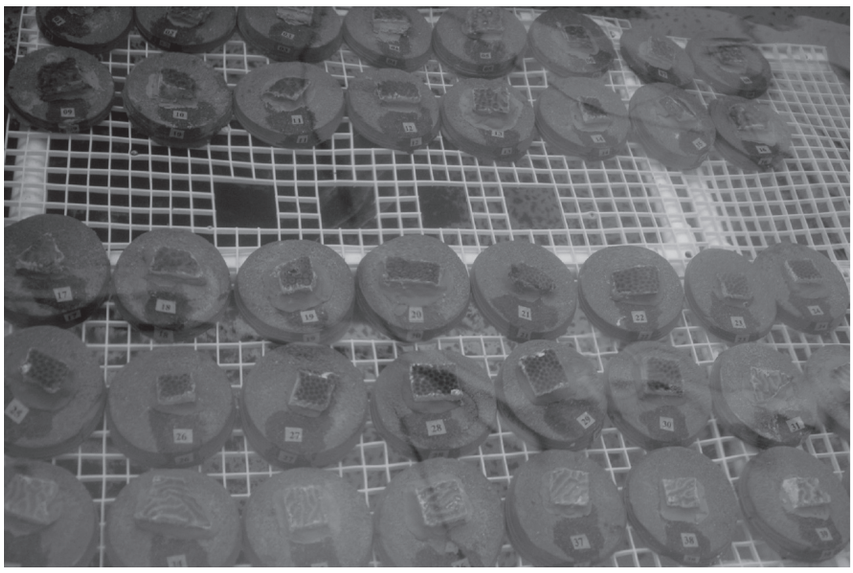

Figure 7: Fragments in culture at the Tropical Aquaculture Laboratory, University of Florida.

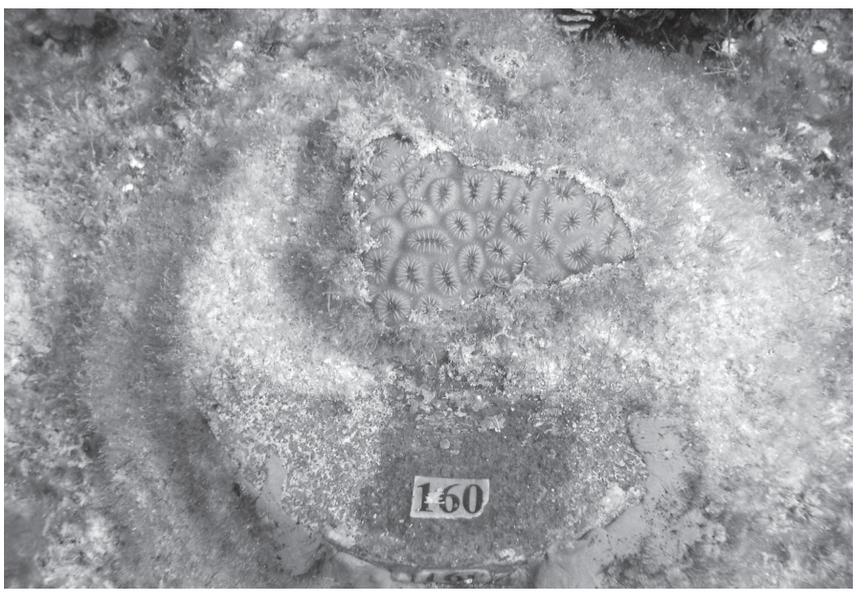

Figure 8: Fragment of Dichocoenia stokesii *after 3 months in the field.*

and a visual health examination was recorded (including an assessment of condition and color), along with any mortality of test fragments.

Caribbean coral health certification process

Prior to the reintroduction of aquacultured coral fragments, a health certification protocol needed to be established. Important considerations included: i) developing working definitions for "diseased" and "healthy" cultured corals, ii) maximizing the potential for cultured corals to survive and thrive in reintroduction sites, iii) minimizing the potential for release of cultured corals with "exotic" disease pathogens or other pathogens of concern into the wild. The goal was to develop a reasonable and practical, health certification process for the State of Florida which could act as a "template" for other studies. But just what is reasonable and practical? There is no such thing as zero risk or 100 % certainty and assessments should be based on present day science, economics, federal and state policies, and reality**.** All protocols should be part of a "living document" (constantly under review and change).

The partners developed protocols leading to health certification in collaboration with NOAA, FKNMS, the United States Department of Agriculture/Animal Plant Health Inspection Service (USDA/APHIS), and FWC. Upon approval, a Special Activity License (SAL) from FWC was also required at the time of reintroduction. Parameters evaluated included visual inspection, water quality testing, biosecurity (for land-based facilities), and histology evaluation and microbial community analysis of aquaculture normals and diseased fragments.

Health assessments on parent colonies were done first at the time of collection (see Appendix A - *Health Assessment Form*), during culture, one month prior to reintroduction for the issuance of the health certificate, and at periodic intervals after being introduced back into the field. Fragments are monitored for visual abnormalities based on the fact that known coral diseases are categorized by visual signs (i.e. Black Band disease, White Spot disease, etc).

Any coral colony in culture showing signs of disease undergoes an extensive work-up including a detailed visual description of the problem, photos, live microscopic and stereoscopic evaluation, microbiology testing and histological evaluation. To develop the list of diseases/syndromes reported in culture conditions further, a *Captive Coral Health Survey* is being sent to other facilities propagating corals. An accurate and comprehensive list of pathogens for corals may prove difficult to compile but evaluation of handling methods, prophylactic treatments, biosecurity protocols, and thorough evaluation of problems are being used to develop a method for screening the population for "health." Considerable precedence already exists for similar aquatic animal health screening certifications (e.g. such as for interstate transport and/or stock enhancement releases of shrimp, bivalves and fish).

Based on the recommendations of the veterinarians involved in this study, standard best management practices have been collaboratively developed to quarantine corals in culture properly and to ensure that corals are healthy prior to reintroduction to restoration sites (Appendix B – *Guidelines*). These procedures and other diagnostic methods were used to develop criteria for issuance of a federal health certification (Appendix C - *Health Certificate*) prior to reintroduction of corals to restoration sites or where otherwise required. The criteria outlined in the health assessment form are used to provide each fragment with a score. Based on the score, fragments either pass or fail inspection. Then, the entire group of fragments of a given species is also assessed. If one fragment out of ten failed, then only that failed fragment would not be transplanted... However, if only one fragment out of ten passed, none of the fragments from that species would be transplanted. The delineation point of whether or not a species get transplanted is 50 %; i.e. half of the inspected coral fragments from a given species need to pass inspection. Failed fragments are then returned to culture and evaluated on a monthly basis. At the time of field visits, those fragments passing inspection would be transplanted to the restoration site following the issuance of appropriate permits.

Diagnostics

In an attempt to support visual observations and to provide a library of normal vs. abnormal changes, histology and microbiological tests have been applied to many of the samples. Samples are obtained for evaluation at the time of collection (from parent colony), in the presence of disease (includes abnormal and normal tissue), prior to reintroduction and the issuance of a health certificate, and during field

sampling if necessary.

Fragments for histology are fixed in a four part seawater to one part buffered zinc formalin (Z-fix concentrate®) solution. They are then immersed in SeaKem® agarose and exposed to vacuum pressure to pull agar into the crevices of the colony. Afterwards, a window is cut into the stiffened agar to expose the skeleton and the specimen is decalcified in neutral EDTA. The remaining tissue, held by the agar in a normal position, is then processed by routine cutting and staining techniques.

Corals rely on a bacteria-laden mucus layer to act as a protective barrier against pathogens and to better adapt to changing environmental conditions. Disease and bleaching in corals are often associated with changes in their microbial community. Identification of various coral diseases and syndromes has classically been limited to visual characterization, but by the time changes are evident the coral is often already compromised. Evaluating microbial communities could therefore help assess the overall health of the fragment. However, less than 1 % of all bacteria present on coral is isolatable in laboratory culture (Ritchie, 2004) and without fulfilling Koch's postulates one cannot attribute disease to a particular isolate. How best then to address changes and potential risks? We have been utilizing the Biolog® system, using EcoPlates™ to evaluate microbial communities, as opposed to individual species. A 96-well culture plate (with 31 carbon sources and a control with 3 replicates per plate) is incubated with mucus from the coral fragment in questions. As the microbes grow, they use a variety of the available carbon sources available in the microwells, resulting in turbidity and color changes in those wells. The Biolog® automated reader then evaluates the plate at two different wavelengths to produce a profile of metabolic activity which provides information on community structure and function. While individual isolates are not identified, we are evaluating the results to see if the process can be developed into a cost-effective clinical tool similar to microbial community analyses used to assess health in other species groups, e.g., ratios of basic bacterial groups obtained from choanal and cloacal (throat) swabs in birds.

Histology and microbiology for the health certification process are currently at the early stages of development, and their use in a reasonable and practical health certification process is uncertain. In future studies, the project team will be utilizing molecular profiling techniques for the detection of changes in the coral-associated bacterial community, before and after disease signs are present.

In sum, it appears that a detailed history, including careful evaluation of adherence to best management practices, and visual inspection by qualified individuals will be most useful for health certification. Currently, several of the veterinarians in this study have been approved by the various state and federal agencies to apply the health certification process to a select group of corals in Florida. These vets are also USDA accredited and can demonstrate experience with coral systems, health and disease.

PROJECT STATUS

In review, 70 fragments (control) from 7 species were planted at the restoration site in May, 2006. Sites for attachment were cleared of encrusting organisms (mainly calcareous algae) and the Z-Spar® epoxy was used to adhere the concrete base. The remaining two sets were cultured for seven months at the two land-based sites: i) Tropical Aquaculture Laboratory (70 fragments) and ii) Mote Maine Laboratory, Summerland Key (70 fragments). Health assessments, both in culture and the field, were conducted every 3 months. The health certificate for the cultured corals was issued within 30 days of planting in order to accommodate the diagnostic tests. In sum, 88 fragments out of 140 passed the certification evaluation. Fragments failed primarily due to *Aptasia* overgrowth at the flow-through system resulting in lower condition scores. Fragments passing the health certification process were then planted in the field during the first week of December, 2006. The current plan is to evaluate all of the planted fragments every three months for a number of years to assess growth and survival of fragments and to compare the success of each culture regime.

Additional funding has been received to: i) continue monitoring the first study set of corals, ii) explore the relationship between mounting techniques and subsequent coral growth and health; iii) further evaluate microbial health of corals in culture and changes when reintroduced to the field using molecular methods for bacterial community analysis; and iv) assess genetic variation among local and regional corals to begin to understand the potential geographic range of coral fragments used in restoration.

The various studies are designed to maximize fragment use, and to coordinate collection and monitoring efforts to minimize time and cost by integrating many of the outstanding questions surrounding the successful use of cultured coral fragments for reef restoration.

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REFERENCES

Berzins, I.K., C. Watson, R. Yanong and K.H. Kilgore, 2007. Use of aquacultured coral fragments for restoration activities in the Florida Keys. Proceedings, 38th Annual Conference of the International Association for Aquatic Animal Medicine, Lake Buena Vista, Florida. pp. 6-7.

Berzins, I.K., S. Coats, R.P.E. Yanong, C. Watson, J. Matysczak and K.Nedimyer, 2005. Coral restoration using colonies derived from aquacultured fragments. Proceedings, 36th Annual Conference of the International Association for Aquatic Animal Medicine, Seward, Alaska. pp. 81-82.

Blankenship, H.L. and K.M. Leber. 1995. A Responsible Approach to Marine Stock Enhancement. American Fisheries Society Symposium, 15:167-175.

Breitbart, M., R. Bhagooli,S. Griffin, I. Johnston, and F. Rowher, 2005. Microbial communities associated with skeletal tumors on *Porites compressa*. FEMS Microbiology Letters, 243:431-436.

Brown, B.E. and J.C. Bythell, 2005. Perspectives on mucus secretion in reef corals. Mar. Ecol. Progr. Ser., 296:291-306.

Clark, S. and A.J. Edwards, 1995. Coral transplantation as an aid to reef rehabilitation: evaluation of a case study in the Maldive Islands. Coral Reefs, 14:201-213

Custodio, H.M. and H.T. Yap, 1997. Skeletal extension rates of *Porites cylindrica* and *Porites (Synaraea) rus* after transplantation to two depths. Coral Reefs, 16:267-268

Ducklow, H.W. and R. Mitchell, 1979. Composition of mucus released by reef coelenterates. Limnol. Oceanogr., 24:706-714.

Kilgore, K.H., R.P.E. Yanong, J.S. Graves, C. Watson and I.K. Berzins, 2007. Characterization of microbial communities within the surface mucopolysaccharide layer of corals via metabolic profiling. Proceedings, International Association for Aquatic Animal Medicine 38th Annual Conference, Orlando, Florida, pp. 203-204.

Kline, D.I., N.M. Kuntz, M. Breitbart, N. Knowlton and F. Rohwer,2006. Role of elevated organic carbon levels and microbial activity in coral mortality. Mar. Ecol. Progr. Ser., 314:119-125.

Lindahi, U., 1998. Low-tech rehabilitation of degraded coral reefs through transplantation of staghorn corals. Ambio, 27(8):645-650.

Matysczak, J., R.P.E. Yanong, I.K. Berzins and S. Coats, 2005. Diagnostics involved in the certification of aquacultured corals for restoration. Proceedings, 36th Annual Conference of the International Association for Aquatic Animal Medicine, Seward, Alaska. pp. 79-80.

Precht, W.F., 2006. Coral Reef Restoration Handbook. 1st edn. CRC Taylor & Francis Group. 363 pp.

Ritchie, K.B., 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar. Ecol. Progr. Ser., 322:1-14.

Ritchie, K.B. and G.W. Smith, 2004. Microbial communities of coral surface mucopolysaccharide layers. Coral Health and Disease. R. E. and Y. Loya. New York, Springer-Verlag, pp. 259-264.

Rohwer, F., M. Breitbart, J. Jara, F. Azam and N. Knowlton, 2001. Diversity of bacteria associated with the Caribbean coral *Montastrea franksi*. Coral Reefs, 20:85-91.

Rohwer, F., V. Seguritan, F. Azam and N. Knowlton, 2002. Diversity and distribution of coral associated bacteria. Mar. Ecol. Progr. Ser., 243:1-10.

Van Treeck, P. and H. Schumacher. 1997. Initial survival of coral nubbins transplanted by a new coral transplantation technology-options for reef rehabilitation. Mar. Ecol. Progr. Ser., 150:287-292.

Yap, H.T., P.M. Alino and E.D. Gomez, 1992. Trends in growth and mortality of three coral species (Anthozoa: Scleractinia), including effects of transplantation. Mar. Ecol.Progr. Ser., 83:91-101.

Yap, H.T., R.M. Alvarez, H.M. Custodio III and R.M. Dizon, 1998. Physiological and ecological aspects of coral transplantation. J. Exp. Mar. Biol. Ecol., 229:69-84.

APPENDIX I: Health assessment form

Coral Health Assessment Form

Fragment Number	Species	Size	Condition	Color	Comments

Condition: 1: dead, 2: <25% of tissue alive, 3: 25-50% of tissue alive, 4: 50-75% of tissue alive, 5: 75-95% of tissue alive, 6: no apparent tissue loss

Color: 1: 100% bleached, 2: partial bleach, 3: lighter than normal, 4: good color

Comment codes: TS: tissue severed, MD: metal deposits present, F: fouling organisms present, CS: cracked skeleton without severed tissue

APPENDIX II: Guidelines (draft)

Guidelines in Preparation for Coral Health Certification

(draft)

Veterinarians involved with the coral health certification process must be USDA accredited, with coral health and disease training and/or experience, and familiarity with the aquaculturists and the culture facility (in veterinary medicine, this is known as establishing a good VCPR (Veterinary/client/patient relationship). The veterinarian should be working with the aquaculturists and facility well in advance of the health certification process.

The following is a list of subject areas with which the veterinarian should be familiar:

1. Collection History
    a. Permitting process for corals
    b. Chain of custody: permit through FKNMS/NOAA
    c. Collection information
        i. Condition (1-6); (1-7 scale used for cultured fragments)
        ii. Color (1-4)
        iii. Disease
        iv. Growth anomaly
        v. Competing algae
        vi. Other organisms

2. Culture Information
    a. System set up/design
        i. Recirculating system
            1. Holding tank configuration structure (L x W x H); water depth;
            2. Filtration:
                a. Biological type (if present)
                b. Mechanical type (if present)
                c. Chemical type (if present)
            3. Water flow rates through holding tank (s)
            4. Surge device configuration/rate
        ii. Flow through
            1. Holding tank configuration structure (L x W x H); water depth;
            2. Water flow rates through holding tank (s)
            3. Surge device configuration/rate
    b. Coral fragment base/attachment configuration/materials
    c. Water quality information/management
        i. Parameters tested/periodicity of testing/acceptable ranges/records of testing:
            1. Temperature
            2. pH
            3. Ammonia, nitrite, nitrate
            4. Phosphate
            5. Hardness
            6. Salinity
            7. Alkalinity
            8. Calcium
            9. Selenium and magnesium (if possible)
            10. Light reading

APPENDIX II (continued): Guidelines (draft)

1. Other parameters/observations should be noted if unusual (e.g., turbidity, color)

i. Management for each parameter above (types of additions/water changes)/records of these

a. History of disease problems
   i. Brief written description of disease events and resolution; include dates; number of fragments affected; species affected

1. Culture Information/History: Biosecurity/water supply

   a. Artificial seawater
      i. Source of freshwater (potential for contamination)
         1. "Protected" source (deep well/spring?)
            a. If not protected/biosecure, type of disinfection used prior to use (methods)
         2. Record of source water testing
            i. Temperature
            ii. pH
            iii. Ammonia, nitrite, nitrate
            iv. Phosphate
            v. Salinity
            vi. Alkalinity
            vii. Calcium
            viii. Selenium
            ix. Magnesium
            x. Other parameters/observations should be noted if unusual (e.g., turbidity, color)
      ii. Seawater mix used: Instant Ocean; Reef Crystals; other?
   b. Caribbean/local (Keys) seawater—should be from same location as corals ("same location" to be defined by FKNMS); documentation
   c. Coral parent colonies will be collected from an area considered "local" to region for stock enhancement (as defined by FKNMS); documentation
   d. Corals from "different locations" in the Keys should not be mixed in culture (FKNMS definition for "different locations")
   e. Documentation required verifying absence of Indo-Pacific (or from any other are) corals/organisms. Only SE Gulf/SW Atlantic or Keys organisms allowed in systems
   f. Presence of competing algae (non-zooxanthellae): filamentous/phytoplankton
      i. Management protocol (including use of chemicals?)
   g. Presence of other organisms
      i. Management protocol (including use of chemicals?)

2. Training and Experience with Corals (through sanctioned training programs/work with sanctioned veterinarians/disease course work and experience)

   a. Familiarity with growth characteristics and normal acceptable variations (growth, color) for species in culture
   b. Familiarity with clinical signs of disease in corals
      i. Common diseases of concern (field and culture)
      ii. Assessment based on tissue condition; color
      iii. Disease diagnostics/sampling methods
      iv. Assessment

APPENDIX III: Health certificate (draft)

Coral Health Certification Protocol

(draft)

Recommendations developed by
The Florida Aquarium and the University of Florida, Tropical Aquaculture Laboratory

1. Collection History

    a. Chain of custody: permit through FKNMS/NOAA
    b. Collection information
        i. Source of coral (e.g., rescue donation, grounding opportunity, permitted collection, or purchased from other source)
        ii. Condition (1-6); (1-7 scale is for fragments)
        iii. Color (1-4)
        iv. Growth anomaly
        v. Other potential disease issues

1. Culture Information/History: Biosecurity/water supply

    a. Artificial seawater
        i. Type/brand
        ii. Source of freshwater (should be approved in advance)
    b. Caribbean/local (Florida Keys) seawater— seawater should be from within the same region of the Florida Keys from which corals were collected (e.g., Upper, Middle, Lower Keys, or Dry Tortugas)
    c. Mean salinity throughout culture period
    d. Photoperiod and type of lighting used throughout the culture period
    e. Documentation required verifying absence of any corals or organisms which are not from the Western Atlantic, Gulf of Mexico, or Florida Keys
    f. Only SE Gulf/SW Atlantic or Keys organisms allowed in systems
    g. Presence of visual diseases or abnormalities
        i. Disease
            1. Description
            2. Diagnostics done?
        ii. Growth anomaly
            1. Description
        iii. Treatment protocol
    h. Presence/extent of competing algae (non-zooxanthellae): filamentous/phytoplankton. Treatments?
    i. Presence/extent of other organisms. Treatments?

2. Visual inspection: Categorical Classification of Coral Health Parameters

Individual Fragments will be assessed, but status of species population as a whole will also be assessed.

If a group of fragments of one species is exhibiting variable health conditions, fragments rated 1-5 condition, 1-2 color, or have other disease conditions should be separated from the remaining colonies for purposes of evaluation; general health history from the aquaculturist of the population will be requested. These fragments will then be assigned a consideration score. This consideration score will be used for long term evaluation of species culturability and culture conditions and will also help avoid release of chronically diseased corals. All

APPENDIX III (continued): Health certificate (draft)

fragments that are assessed to be "not acceptable, not releasable at this time" will be allowed to remain in culture for potential re-introduction and re-assessment if their consideration score is 2 or greater.

CRITERIA/SCORES (The 3 Cs)

a. Condition score

1- Dead
2- < 25% of tissue alive
3- 25-50% of tissue alive
4- 50-75% of tissue alive
5- 75-95% of tissue alive
6- No apparent tissue loss
7- Growth over formerly dead tissue/ Overgrowth over edges

b. Color score

1- 100% bleached
2- Partial bleach
3- Lighter than normal
4- Good color

c. **Consideration score**

1- Failed 10 assessments
2- Failed 7-9 assessments
3- Failed 4-6 assessments
4- Failed 1-3 assessments
5- First assessment

1. Overall Assessment of Corals: Population Assessment/Acceptable Parameters

    a. Evidence of growth/thriving in culture/acceptable tissue condition: condition score 6 or 7
    b. No significant evidence of bleaching: color score 3 or 4
    c. Consideration score: 2 or greater is acceptable;
        i. Consideration score of 1 will result in fragment designation of "not acceptable, not releasable, permanent status" (see section 6 below)
    d. No evidence of significant other visual diseases

2. Verification of Regional Collection and Re-Introduction Compatibility

    a. Verification required that coral will be re-introduced to the field within the same region of the Florida Keys from which they were collected (either Upper, Middle, and Lower Keys, or Dry Tortugas), unless compelling scientific information on genetic composition of any given coral species dictates otherwise

3. Disposition

    a. Based upon history, adherence to biosecurity, origin of parent colonies, water supply requirements, visual inspection (with possible diagnostic and microbiological support) stocks will either be deemed: 1) acceptable and healthy for release; 2) not acceptable, not releasable at this time; or 3) not acceptable, not releasable, permanent status

APPENDIX III (continued): Health certificate (draft)

i. Acceptable and healthy for release
    1. Fragments will have to be released within 30 days from evaluation; any changes in health status in this time period should be reported to FKNMS prior to release for consideration
ii. Not acceptable, not releasable at this time
    1. This will be based on assessment/scores, overall in culture species population status, and information on species culture growth characteristics
    2. Description of disease problem (this can be categorical based on assessment sheet and can have percentage tissue affected for each frag) and/or reason for rejection
    3. FKNMS to provide guidance on disposition
iii. Not acceptable, not releasable, permanent status
    1. Evidence of major disease issue (especially presence of “field disease type” clinical signs) may result in not releasable, permanent status
    2. Description of disease problem (this can be categorical based on assessment sheet and can have percentage tissue affected for each frag) and/or reason for rejection
    3. FKNMS to determine disposition